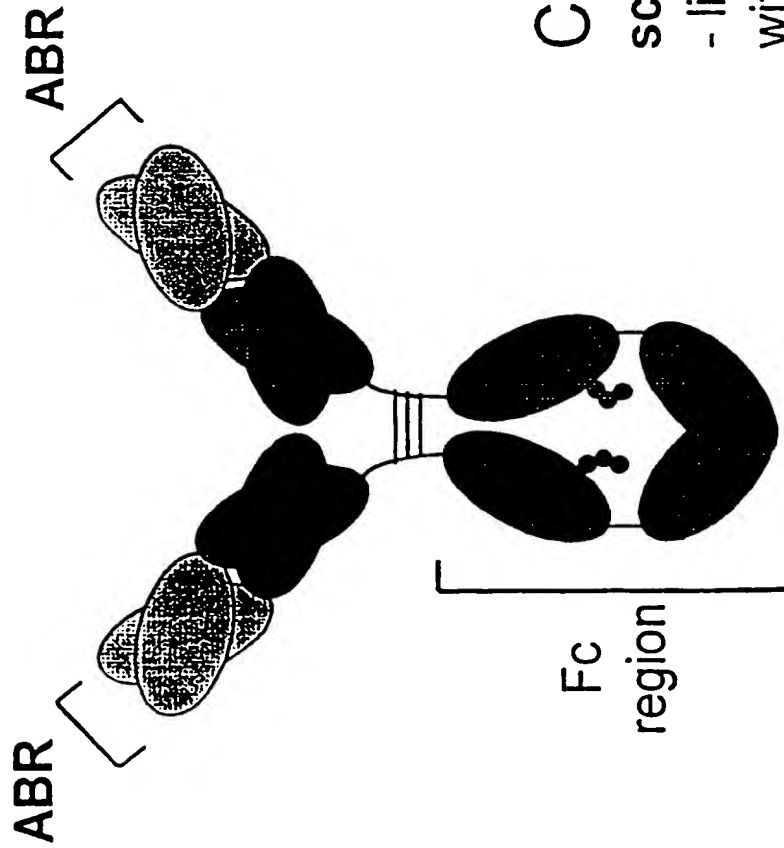


# IgG and binding Fragments

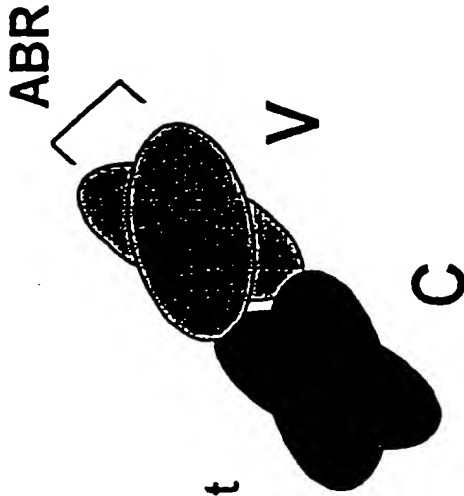
A) Intact IgG



B)

Fab fragment

- $V_L C_L$
- $V_H C_H$
- S=S linked



C)

scFv fragment

- linked V regions with synthetic linker  $(Gly_4Ser)_3$

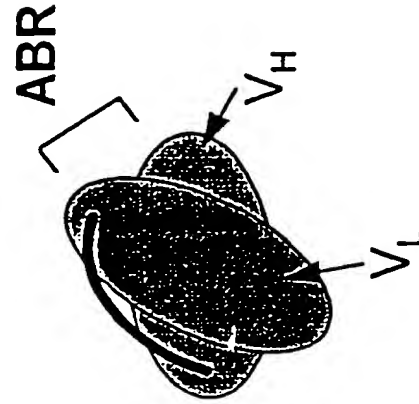


Figure 1

# Recombinant Positive Control Reagent

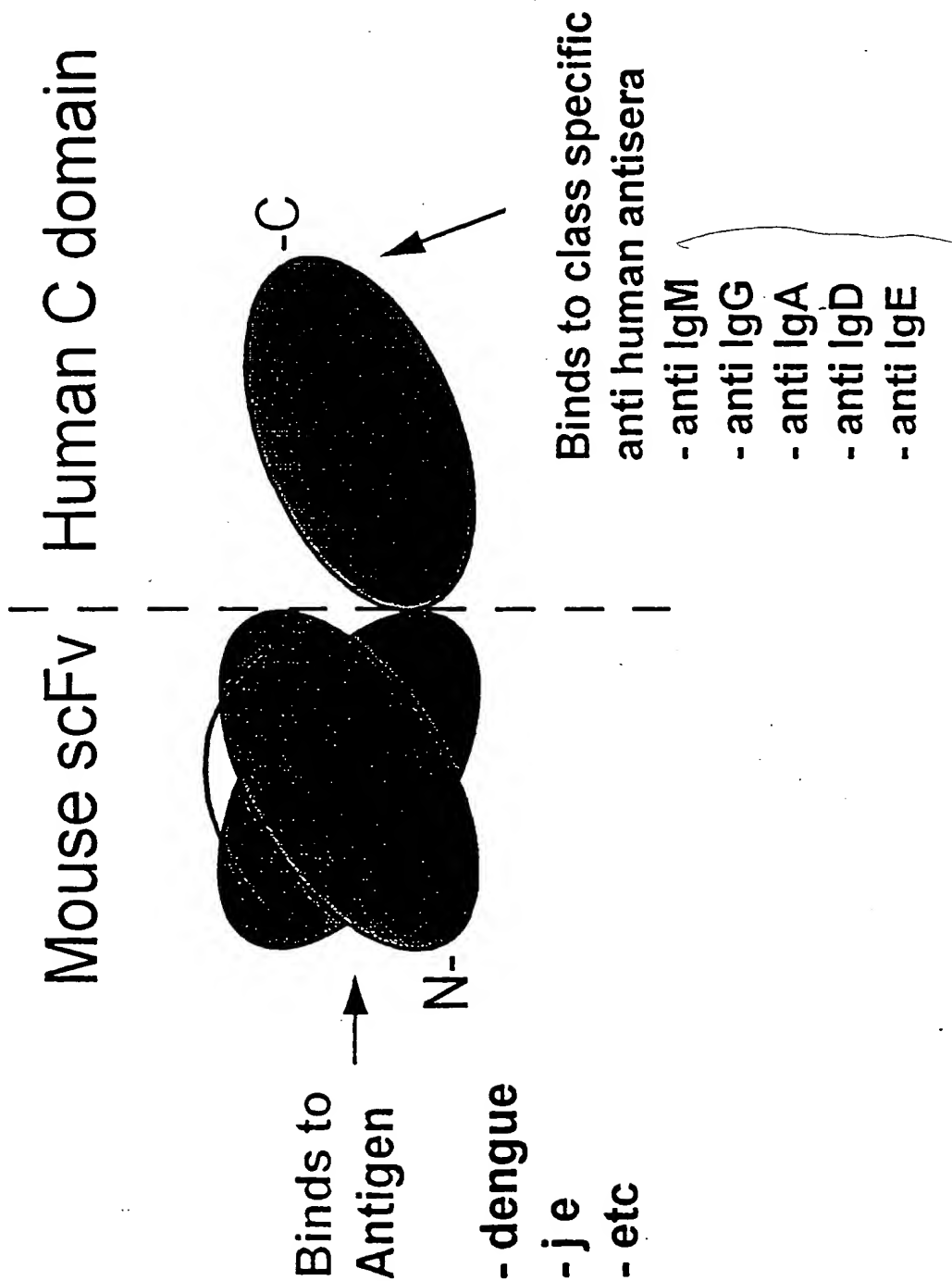


Figure 2

Figure 3

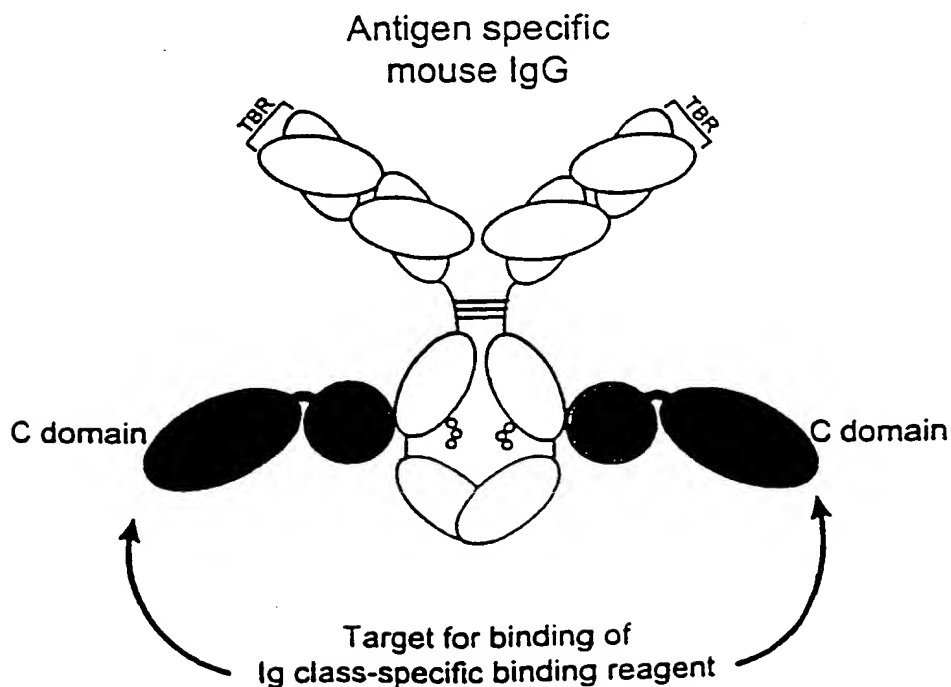
Region 1

Region 2

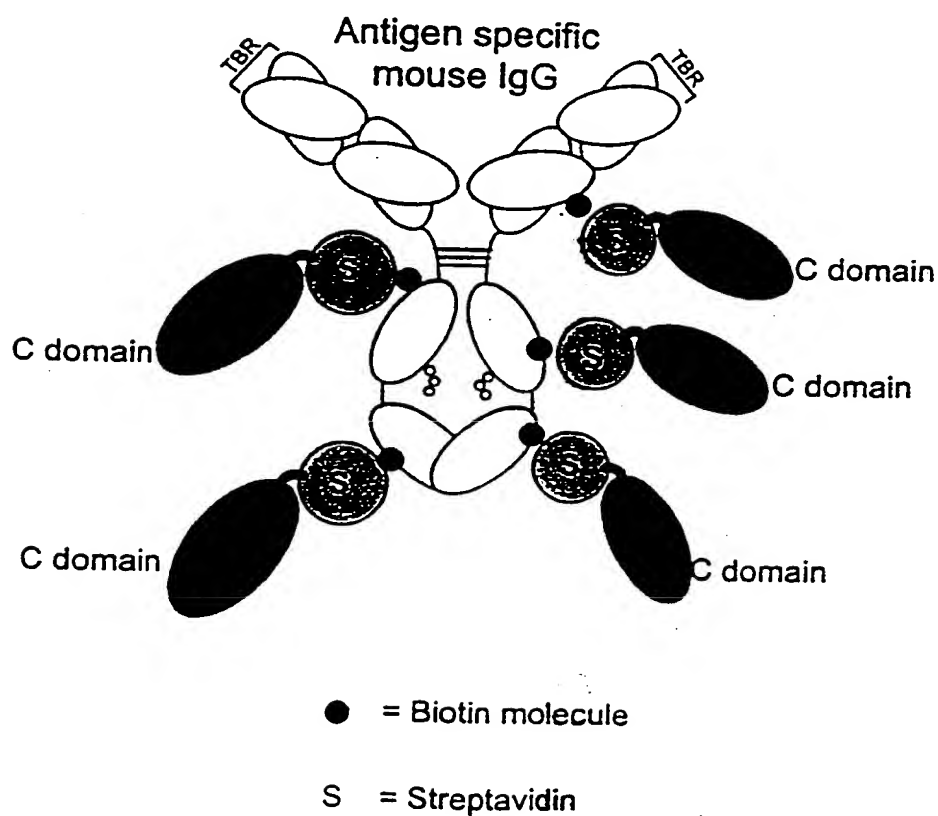
binds to  
Mouse IgG

Immunoglobulin C domain  
reactive to class specific  
anti immunoglobulin binding reagent

Complex formed between bifunctional  
molecule and mouse IgG

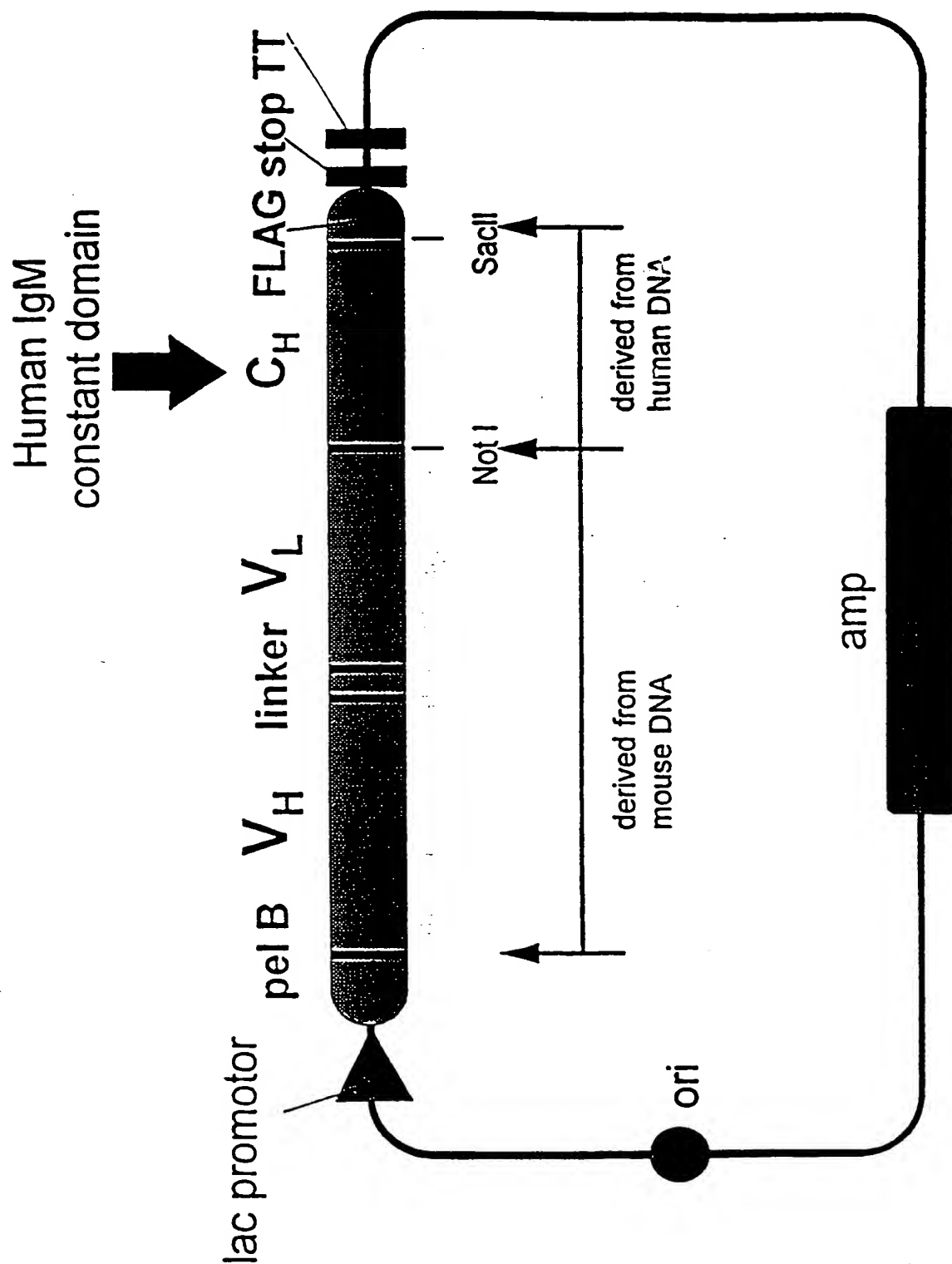


**Complex formed between bifunctional  
molecule containing streptavidin  
and biotinylated mouse IgG**



005790"426T8560

Figure 5



006T90"426T8560

6/11

Figure 6

# ELISA reactivity of 1C3- $\mu$ domain chimeras

Glycophorin on Plate: Probed with sheep anti Human IgM HRP

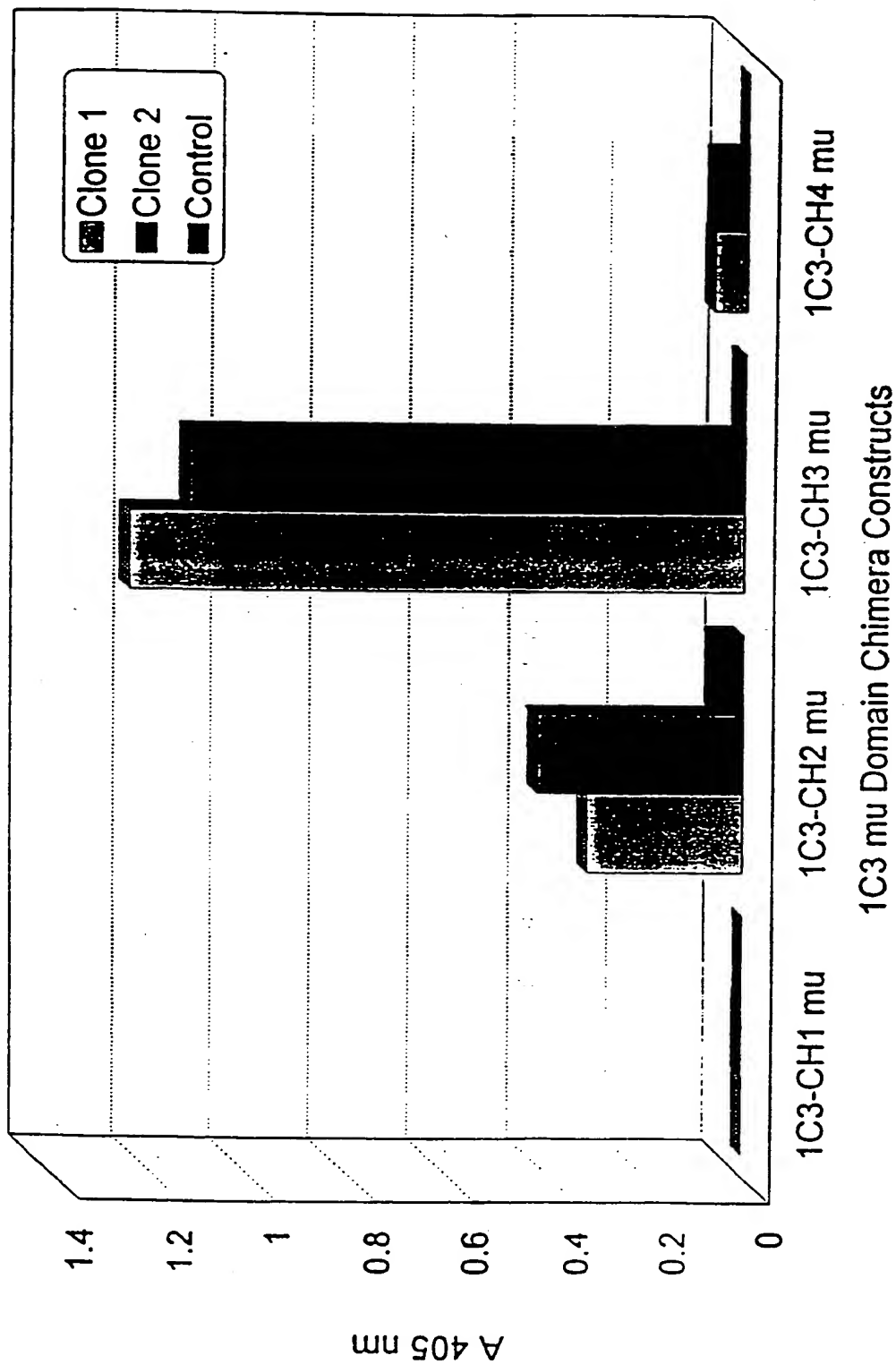


Figure 7

# Dengue IgM Capture ELISA

Reactivity of 13C11-CH3 $\mu$  protein from *E coli* TOPP6 periplasm

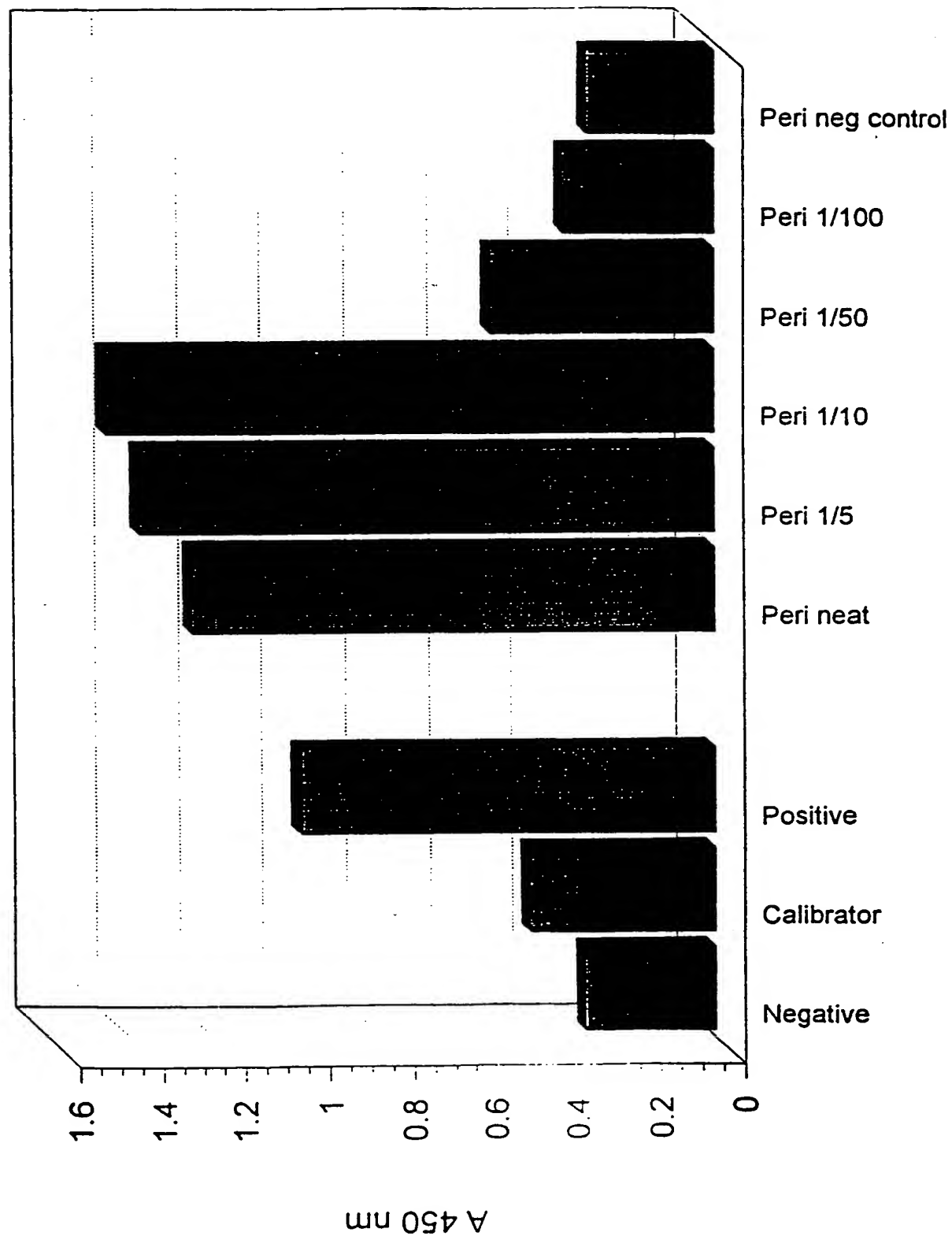


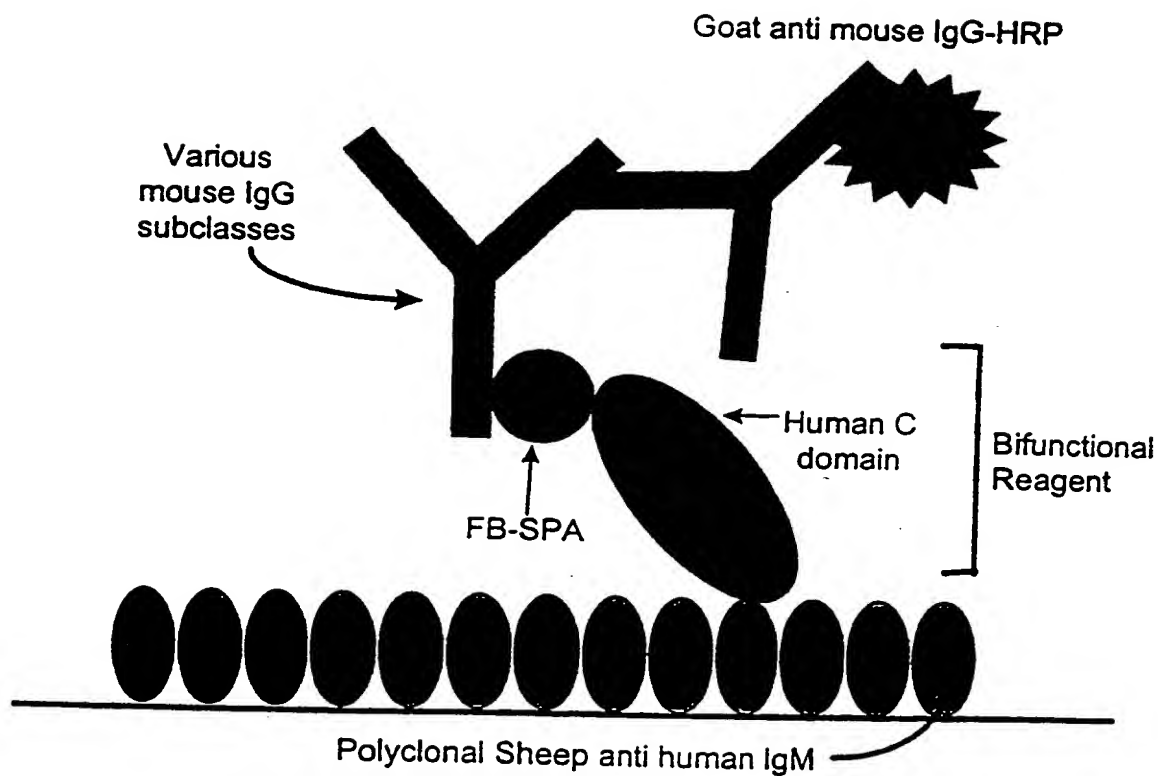
Figure 8

2353 M K Y L L P T A A A G L L L L A  
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG  
 \--- Pel B-- --->  
 2401 A Q P A M A A D N K F N K E Q Q  
 GCC CAG CCG GCC ATG GCC GCG GAT AAC AAA TTC AAC AAA GAA CAA CAA  
 --- Sfi 1 <N col > < Start Fragment B  
 2449 N A F Y E I L H L P N L N E E Q  
 AAT GCT TTC TAT GAA ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA  
 2497 R N G F I Q S L K D D P S Q S A  
 CGC AAT GGT TTC ATC CAA AGC CTA AAA GAT GAC CCA AGC CAA AGC GCT  
 2545 N L L A E A K K L N D A Q A P K  
 AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GAT GCT CAA GCA CCA AAA  
 End Fragment B->  
 2593 S D P A A A D Q D T A I R V F A  
 AGT GAT CCC GCG GCC GCA GAT CAA GAC ACA GCC ATC CGG GTC TTC GCC  
 < linker > NotI > CH3 mu domain  
 2641 I P P S F A S I F L T K S T K L  
 ATC CCC CCA TCC TTT GCC AGC ATC TTC CTC ACC AAG TCC ACC AAG TTG  
 2689 T C L V T D L T T Y D S V T I S  
 ACC TGC CTG GTC ACA GAC CTG ACC ACC TAT GAC AGC GTG ACC ATC TCC  
 2737 W T R Q N G E A V K T H T N I S  
 TGG ACC CGC CAG AAT GGC GAA GCT GTG AAA ACC CAC ACC AAC ATC TCC  
 2785 E S H P N A T F S A V G E A S I  
 GAG AGC CAC CCC AAT GCC ACT TTC AGC GCC GTG GGT GAG GCC AGC ATC  
 2833 C E D D W N S G E R F T C T V T  
 TGC GAG GAT GAC TGG AAC TCC GGG GAG AGG TTC ACG TGC ACC GTG ACC  
 2881 H T D L P S P L K Q T I S R P K  
 CAC ACA GAC CTG CCC TCG CCA CTG AAG CAG ACC ATC TCC CGG CCC AAG  
 2929 G A A D Y K D D D D K \*  
 GGc GCC GCG GAT TAT AAA GAT GAT GAT GAT AAA TAA GAA TTC AGC CCG  
 Sac 2 ----- FLAG ----- Eco RI <-----  
 2977 CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT TTT ACA ACG  
 ----- TrpA terminator ----->



9/11

Figure 9



09581924, 061900

10/11

Figure 10

Sequence of expression cassette Str-C<sub>H</sub>3μ in pGC vector

5 M K Y L L P T A A A G L L L L A  
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG  
 |--- Pel B-- -->  
 A Q P A M A E A G I T G T W Y N  
 GCC CAG CCG GCC ATG GCC gag gcc ggc atc acc ggc acc tgg tac aac  
 10 --- Sfi I <Nco I >-----core streptavidin ----->  
 Q L G S T F I V T A G A D G A L  
 cag ctc ggc tcg acc ttc atc gtg acc gcg ggc gcc gac ggc gcc ctg  
 T G T Y E S A V G N A E S R Y V  
 15 acc gga acc tac gag tcg gcc gtc ggc aac gcc gag agc cgc tac gtc  
 L T G R Y D S A P A T D G S G T  
 ctg acc ggt cgt tac gac agc gcc ccg gcc acc gac ggc agc ggc acc  
 20 A L G W T V A W K N N Y R N A H  
 gcc ctc ggt tgg acg gtg gcc tgg aag aat aac tac cgc aac gcc cac  
 S A T T W S G Q Y V G G A E A R  
 25 tcc gcg acc acg tgg agc ggc cag tac gtc ggc ggc gcc gag gcg agg  
 I N T Q W L L T S G T T E A N A  
 atc aac acc cag tgg ctg ctg acc tcc ggc acc acc gag gcc aac gcc  
 W K S T L V G H D T F T K V K P  
 30 tgg aag tcc acg ctg gtc ggc cac gac acc ttc acc aag gtg aag ccg  
 S A A S D P A A A D Q D T A I R  
 tcc gcc gct agc gat ccc gcg gcc gca gat caa gac aca gcc atc cgg  
 strep-| -----< linker > <-Not I > |-----C<sub>H</sub>3μ domain  
 35 V F A I P P S F A S I F L T K S  
 gtc ttc gcc atc ccc cca tcc ttt gcc agc atc ttc ctc acc aag tcc  
 T K L T C L V T D L T T Y D S V  
 40 acc aag ttg acc tgc ctg gtc aca gac ctg acc acc tat gac agc gtg  
 T I S W T R Q N G E A V K T H T  
 acc atc tcc tgg acc cgc cag aat ggc gaa gct gtg aaa acc cac acc  
 N I S E S H P N A T F S A V G E  
 45 aac atc tcc gag agc cac ccc aat gcc act ttc agc gcc gtg ggt gag  
 A S I C E D D W N S G E R F T C  
 gcc agc atc tgc gag gat gac tgg aac tcc ggg gag agg ttc acg tgc  
 50 T V T H T D L P S P L K Q T I S  
 acc gtg acc cac aca gac ctg ccc tcg cca ctg aag cag acc atc tcc  
 R P K G A A D Y K D D D D K \*  
 55 cgg ccc aag ggc gcc gcg gat tat aaa gat gat gat gat aaa taa GAA  
 Sac 2 |-----FLAG-----| Eco  
 TTC AGC CCG CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT

09581924-061500

Size exclusion FPLC (Superdex200) of  
refolded Streptavidin- $C_H3\mu$

